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EFFECTS OF VIGIL ON HUMAN CIRCADIAN RHYTHMS:

NORMATIVE DATA

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### NORMATIVE DATA \*

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One of the characteristics of circadian cycle is its persistence as a rhythm for two or more cycles after elimination of the environmental cycle<sup>17</sup> and sleep. Sleep loss can be used as a test to examine whether a given rhythmic event is circadian, or not. Aschoff and his associates<sup>2,3</sup>, Froberg and others<sup>10</sup>, Murray and others<sup>18</sup>, and Weber<sup>28</sup> observed that some rhythms in biochemical, physiological and psychological activities persisted after disturbed sleep, or after total sleeplessness up to 75 hours.

Strong circadian rhythms, such as body temperature, cortisol secretion, have not been shown to be seriously disturbed by vigil for one or two nights. But sleep loss affected them very subtly to result in altered acrophase angle, in diminished strength of rhythmicity, and perhaps in weakened synchronization with other circadian variables.

Effects of total sleep loss of two nights on circadian cycles have, however, not yet examined systematically with the cosinor method. The purpose of this study was to see how circadian rhythms of some variables changed their acrophase angles and strength of rhythmicity as a result of vigil lasting up to 45 hours.

#### MATERIALS AND METHODS

As part of a larger experiment study of "recuperative" power of naps from fatigue due to sleep loss, a total of 23 male naval subjects in two groups of 15 (average age of 21.5, 18-30) and 8 (average age of 18.6, 18-21) completed the experimental schedule (see Fig. 1). The experiment required subjects to live, two at a time, in a sleep laboratory over six consecutive days. During Sunday and the first baseline

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Figure 1 About Here  
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day (Monday), subjects were instructed on how to measure oral temperature, pulse, and how to complete a variety of adjective checklists. They were also trained on psychological tasks during Sunday and Monday.

Some performance tasks were expected to show improvement due to practice effects over task sessions, while others could show diminished performance due to fatigue effects over task sessions. These effects confound the circadian cycle, if there is any, in task performance. Usually Latin Square or other experimental designs are used so that data at each timepoint in the day were represented by the performance scores of all possible test sessions<sup>5,6</sup>. These designs, however, required the subjects return to the laboratory over many weeks. Since the subjects in this study were assigned to only one week duty in our laboratory, intensive trainings on performance tasks to asymptotic levels were used to avoid, as much as possible, confounding of practice with circadian effects.

Baseline data included 11 data points from the 8th data collection session (Bio 8 of Fig. 1) to the 18th recording session (Bio 18), covering the time period from 0800 to 0400 (1 to 25 hours continuous wakefulness). Sleep deprivation data included 11 data points covering a period from Bio 20 to the Bio 30 session (Fig. 1), covering the time from 0800 to 0400 (25 to 45 hours vigil).

The following variables are discussed in this paper: oral temperature in °F, systolic and diastolic blood pressures in mmHg, pulse in beats per min, Neuropsychiatric Research Unit (NPRU) Mood Scale in positive and negative scores, Stanford Sleepiness Scale (SSS), School of Aerospace Medicine (SAM) Subjective Fatigue Checklist, Two-Response Alternation Performance (TRAP), Memory and Search Task (MAST), and 4-Choice Serial Reaction Time task.

The positive score on the NPRU Mood Scale is the sum of the response weights assigned to each of 19 positive affect adjectives (such as, active, alert, carefree and others), and it generally decreases with sleep loss. The negative score is the sum of the response weights of 10 negative affect adjectives (such as, annoyed, defiant, drowsy, and others), and it increases with sleep loss. This NPRU Mood Scale was successfully used by Opstad and others<sup>22</sup>. Additional scoring details are given by Johnson and Naitoh<sup>15</sup>. The SSS was developed by Hoddes and others<sup>13</sup> to measure sleepiness on a 7 point scale, from "feeling active and vital; alert; wide awake" (one point) to "almost in reverie; sleep onset soon; losing struggle to remain awake" (7 points).

The SAM Subjective Fatigue Checklist was developed by Pearson and Bayars<sup>23</sup>, and has been used extensively to measure fatigue among air-crews (for instance, a study by Hale and his colleagues<sup>12</sup>) and also under atypical demanding work-rest schedules (see, for example, Storm and others<sup>24</sup>). The score of the SAM Fatigue Checklist is the sum of the weights given to each of ten statements, such as 'extremely tired', to which each subject chose one of three categories to describe his (or her) feeling best state. The total score ranged from 0 to 20 points, where the lower score indicated a presence of feeling of greater fatigue.

As a simple psychomotor task, subjects did a 6 min task of tapping two response keys, placed 4 cm apart on a sloping face of a task box, alternately at a self-selected steady pace, with eyes closed, and wearing a headphone. If the subject failed to press the response buttons (or pressed the same response button twice, or held down both the response buttons) within 2.5 seconds, an alerting buzzing noise was given to the

subject through a headphone until proper response was made. The device generated 1 msec pulses continuously, and proper response button presses stopped generation of these pulses. These pulses and responses were recorded on cassette tapes. A PDP12 computer detected the stoppage of the pulses and measured elapsed time between the last stoppage of the pulses and the current stoppage of the pulses to measure the inter-response intervals (IRI) in tens of milliseconds. The cassette recorded IRIs were analyzed further with a PDP 12 to obtain two measures of TRAP: 1) a total number of responses (TRAP 1 measure), and 2) average 10 percentile of the slowest IRIs during the last one minute duration of 6 min task (TRAP 2 measure). This task was used recently by Friedmann and her associates<sup>9</sup> in evaluating the effects of gradual sleep reduction on task performances.

A task of Memory and Search Task (MAST) was devised by Folkard and others<sup>17</sup> and has been used in shiftwork studies. The task consisted of searching line after line through lines of 20 letters, printed out on a sheet of paper, for the lines containing letters of a specified target. The target letters (2, 4 or 6 letters) are listed at the top of each test sheet. Subject was asked to place a check mark along the line containing the target letter, and an X mark for the remainder. The memory load of the task was determined by the number of letters in the target. The task lasted 2 mins. The MAST task was scored for total number lines scanned in 2 min (which included both correctly done and incorrectly evaluated lines). Only the results of MAST with 2 letter targets (2 MAST) are reported here, as the MAST tasks with 4 or 6 letters yielded similar results.

The Wilkinson and Doughton<sup>20</sup> portable four-choice serial reaction

time task was also administered. The 4-choice task required the subject to press one of four buttons arranged at the corners of a square, corresponding to a similarly arranged light array directly above the response keys. Lights illuminated them at random, 120 msec following a button press. Subjects were instructed to press the buttons as quickly and as accurately as possible. The 4-choice task was mechanized and housed in a modified portable cassette recorder, and subject's performance was recorded on a cassette tape. Task duration was 6 min. A PDP12 computer obtained minute-by-minute tally of total number of responses, IRIs and errors. In this study the total number of responses during the first 5 min period of task and the 10 percentile of the slowest IRIs during the last min of 6 min task were analyzed.

To determine circadian rhythmicity, the record from each subject was fitted to a 24 hour/cycle sinusoidal wave, using the method described by Naitoh<sup>20</sup>. Cosinor plots for the group data were then made with calculations of confidence intervals for acrophase angles and amplitudes<sup>21</sup>.

To determine if the vigil caused a shift in acrophase angles, the differences in the acrophase angles were evaluated by the Rayleigh test<sup>4</sup>. Also, zero-mu  $t$  tests were used to evaluate the changes in the percent rhythm and in amplitude from the baseline value to the vigil value.

As a preliminary step for understanding the alteration in inter-relations between variables, product-moment correlations were computed between oral temperature and some selected variables. Within-subject correlations were computed under the baseline condition, then another set of within-subject correlations were computed for the vigil condition. Since each correlation was based on within-subject data, usual significant test could not be applied. However, if there exists non-zero

correlation between two variables in a population, then the average over many subjects can be tested for significant difference from zero. Also, the difference between correlation coefficients observed under the baseline and those under the vigil was calculated for each subject, and then the set of differences was tested with zero-mu  $t$  test to see if there was a significant change in magnitude of correlation between the baseline and the vigil. The Dunn-Bonferroni criterion<sup>8</sup> was used to correct level of significance (5% ; two tails).

## RESULTS

Table 1 shows a summary of cosinor analysis, and Table 2 lists the

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Tables 1 and 2 About Here  
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results of statistical tests of changes in the percent rhythm and amplitude of some selected variables from Table 1. All variables exhibited statistically reliable rhythm during the baseline. However, rhythms in diastolic blood pressure, TRAP 2, 4-choice, and in the total number of lines scanned in two min on the MAST are lost during the vigil period, suggesting that these measures are dependent on sleep for their rhythmicity. Vigil did not increase strength of rhythmicity for any variables.

Change in circadian amplitude due to the vigil was not significant for all variables in this study, except TRAP1 which showed a greater amplitude during the vigil than during the baseline period. But that change was not significant with a conservative test.

The most dramatic change in this study was that the 95% confidence ellipse prepared for each variable was invariably much larger during the vigil when compared with the baseline. It was also found that the vigil



shifted significantly TOP of circadian rhythms only in physiological and mood variables. The Rayleigh test shown in Table 1 suggests that none of task performance measures showed such significant shift in acrophase angles. These acrophase shifts were small. For example, TOP of oral temperature was 41 min earlier on the average during the vigil; similarly 31 min phase advance for systolic blood pressure, 27 min delay for TOP of NPRU Positive Scale, 28 min delay in TOP for NPRU Negative Scale, 63 min delay in SSS, and 64 min delay in SAM Subjective Fatigue Checklist - all of these changes as a result of remaining awake up to 45 hours. Changes in acrophase angles were all within two hours, sampling interval used in this study.

Calculation of the percent range of change (%ROC) in Table 1 was based on average mesor and average amplitude, not on individual subjects. The %ROC was obtained by dividing average group mesor by average group amplitude and then multiplying the results by 100. %ROC describes the percentage of circadian modulation of each variable. For instance, oral temperature revealed very small %ROC of 0.6 during the baseline, reflecting that it will be as much as 0.6% (of its mesor) higher or lower from the mesor, while a %ROC for "NPRU Negative Score" would be as much as 45.7% above and 45.7% below the mesor depending on time of day. The vigil appeared to reduce the %ROC.

Intercorrelations between oral temperature and some selected variables are shown in Table 3. During the baseline day, oral temperature correlated

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Table 3 About Here  
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significantly with pulse, SSS, NPRU-, NPRU+, TRAP 1 and TRAP2 measures.

The vigil did not change intercorrelations between temperature and pulse and mood measures, but oral temperature was no longer significantly correlated with TRAP task measures.

#### DISCUSSION

Body temperature, self-rated arousal, and the excretion of both adrenaline and melatonin showed persistent circadian cycles over 75 hours of vigil<sup>1,10</sup>, but circadian rhythmicity in noradrenaline disappeared. Shooting performance seemed to have shown a greater circadian activity as the hours of sleeplessness continued to 75 hours<sup>1,10</sup>. Medd and others<sup>16</sup> observed similar persistence of circadian rhythmicity in body temperature, time estimation, short-term memory, and mood, concluding that a 24 hour vigil had minimal effect on circadian cycle of performance. Cutler and Cohen<sup>7</sup> observed in one night of vigil that circadian cycle in SSS and body temperature continued with a period of "euphoria, increased activities, talkativeness and interaction among the subjects between 4:30 a.m. and 6:00 a.m.".

Aschoff and his associates<sup>2,3</sup> and Weber<sup>25</sup> reported that vigil of up to two nights did not seriously affect circadian cycles, and that interrupted sleep did not result in a significant distortion of circadian cycle in body temperature. By inspecting plots of averages across time of day, they observed that vigil reduced amplitude of the average plot of rectal temperature, time estimation and tapping rate and further that nightly fall was smaller when subjects remained awake. Time-of-trough came three hours earlier for tapping and grip strength tasks. Since none of these studies examined rhythmicity of individual subjects with the cosinor method, it is hard to tell whether a reduction in amplitude, of average rectal temperature plot was caused by actual reduction in amplitude, or

by an increased scatter of the TOP of individual subjects<sup>3</sup>. In fact, Murray and others<sup>18</sup> found no reduction in amplitude of oral temperature circadian rhythm, but an increase during the course of 98 hours of vigil. Medd and others<sup>16</sup> observed no change in temperature cycle in 24-hour sleep loss.

Table 1 shows that the most significant effect of sleeplessness of up to 45 hours was lengthening of 95% confidence intervals for acrophase angles. Thus, for oral temperature, significant changes in circadian cycle were reduced rhythmicity and much larger 95% confidence ellipse. Thus, a major effect of the vigil on the circadian cycles was to create a greater variability in TOP and TOT among the individual subjects. A reduced amplitude seen in a plot of average body temperature and others during the vigil might be due more to a greatly increased individual difference in acrophase angles.

The results of this study extend the findings of Moses and her associates<sup>19</sup> in confirming relation between SSS and oral temperature during the baseline and vigil. However, this study did not find significant correlation between oral temperature and task performance during vigil, probably due to differences in tasks.

#### SUMMARY

A vigil of up to 45 hours does not destroy circadian cycles in physiological and subjective mood scales, but some rhythms in task performances disappeared with vigil. Vigil does, however, alter some basic parameters of circadian rhythmicity. It tends to reduce strength of rhythmicity and most importantly, to create lengthening of 95% confidence arc for acrophase angles, resulting in appreciably larger 95% confidence ellipse. Thus, major altering effects of vigil of two nights seem to be in a

greater scatter of acrophase angles among the individual subjects. Such a greater scatter in time of minimum and maximum due to vigil would result in reduction of amplitude when averages across subjects were plotted along time of day, and also in failure to find a significant cosinor for a group, even though individual subjects might have somewhat reduced but still significant rhythmicity. Relationship between oral temperature and subjective mood and pulse seems to continue undisturbed during the vigil, but correlation between oral temperature and task performance did not survive a process of remaining awake. Findings suggest that more attention must be paid to individual susceptibility to vigil in circadian cycle study.

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Table 1 - Cosinor summary of circadian rhythm during Baseline (B) and Vigil (V) periods.

Variable	Epoch	N	PR	Mesor (SD)	Amplitude Mean(95% C.I.)	Acrophase(hrs min) Mean(95% C.I.)	%ROC	Rayleigh Zd
Oral Temp (°F)	B V	23 23	54.5% 43.5%	97.8(0.3) 97.5(0.5)	0.5(0.4 to 0.7) 0.4(0.3 to 0.5)	1722(1614 to 1819) 1641(1504 to 1813)	0.6% 0.4%	13.6***
Blood Press. Systolic (mmHg)	B V	19 19	38.3% 51.2%	120.3(7.5) 120.4(6.4)	6.9(3.8 to 10.0) 9.0(5.6 to 12.5)	1037(0856 to 1234) 1006(0902 to 1119)	5.8% 7.5%	10.7***
Blood Press. Diastolic	B V	19 19	27.7% 29.9%	71.3(6.4) 70.2(6.2)	2.7(1.0 to 4.6) 1.8	0420(0048 to 0605) 0550	3.8% ----	1.4
Pulse (beats/min)	B V	19 19	31.5% 37.5%	63.7(5.7) 65.0(5.4)	2.8(0.7 to 5.2) 3.3(1.3 to 5.4)	1503(1032 to 1736) 1525(1306 to 1702)	4.4% 5.1%	2.8
APRU +	B V	23 23	47.3% 31.7%	31.9(7.4) 27.5(8.9)	4.0(2.3 to 5.7) 3.2(1.4 to 5.2)	1432(1231 to 1623) 1500(1328 to 1722)	12.6% 11.7%	10.8***
APRU -	B V	22 22	48.6% 39.2%	3.5(2.8) 6.8(4.0)	1.6(0.8 to 2.5) 1.6(0.8 to 2.5)	0327(0206 to 0438) 0355(0154 to 0638)	45.7% 23.6%	6.1**
SSS	B V	22 22	55.7% 35.7%	2.3(0.5) 3.3(0.8)	0.7(0.5 to 1.0) 0.5(0.3 to 0.8)	0236(0132 to 0321) 0339(0138 to 0538)	31.3% 16.1%	8.8***
SAM Fatigue	B V	23 23	56.1% 35.3%	12.4(1.6) 9.8(2.6)	2.1(1.3 to 3.0) 1.6(1.0 to 2.7)	1407(1255 to 1504) 1511(1308 to 1728)	17.0% 16.4%	5.6**
TRAP1 # Responses)	B V	19 19	36.7% 32.6%	1033.6(237.0) 1006.9(247.0)	43.8(24.3 to 66.0) 45.7(4.35 to 88.2)	1946(1741 to 2234) 1707(1208 to 2120)	4.3% 4.6%	2.5
TRAP2 (msec)	B V	19 19	33.2% 24.2%	503.9(151.9) 798.9(372.9)	70.0(29.6 to 121.3) 68.7	0637(0410 to 1035) 0623	13.9% -----	1.0
MAST # Lines)	B V	19 19	25.5% 24.1%	57.2(15.1) 53.1(15.0)	4.3(1.3 to 7.3) 2.5(0.2 to 4.9)	1516(1319 to 1741) 2045(1931 to 0056)	7.5% 4.8%	2.0
Choice # Responses)	B V	8 8	36.8% 10.2%	547.8(59.7) 489.5(85.5)	19.1(4.8 to 42.2) 3.6	1837(1445 to 2353) 0701	3.5% -----	1.1

Table 2 - Changes in percent rhythm ( $R^2$ ) and amplitude of circadian activity due to vigil of 25 to 45 hours.

Variable		Mean Difference between Baseline and Vigil	N	Exact probability for observed difference (%)
Oral Temp.	$R^2$	11.05	23	3.9% <sup>†</sup>
	Amp	0.10	23	8.3%
Systolic Blood Press.	$R^2$	-12.93	19	11.8%
	Amp	- 1.50	19	30.6%
NPRU+	$R^2$	15.54	23	5.0% <sup>†</sup>
	Amp	1.18	23	12.8%
SSS	$R^2$	20.02	22	0.4%* <sup>†</sup>
	Amp	0.08	22	39.0%
SAM Fatigue	$R^2$	20.73	23	0.5% <sup>†</sup>
	Amp	0.10	23	82.1%
TRAP1	$R^2$	4.13	19	45.3%
	Amp	-33.7	19	2.9% <sup>†</sup>
2MAST	$R^2$	1.34	19	80.0%
	Amp	0.15	19	90.0%

\* = Significant at 5% or better (2 tails) with the Dunn-Bonferroni criterion for computation of 14  $t$ -ratios. † = Conventionally significant at 5% or better (2 tails). N = Number of subjects.



Table 3 - Product moment correlations between oral temperature and some selected variables.

	Systolic BP	Diastolic BP	Pulse	SSS	NPRU+	NPRU-	TRAP1	TRAP2	4 Choice TotalR	4 Choice Slow IRI
Baseline										
Mean	.067	-.125	.337*	-.298*	.294*	-.307*	.298*	-.239*	.162	.001
SD	.330	.347	.325	.316	.298	.386	.304	.287	.298	.191
N	19	19	19	22	23	22	19	19	8	8
p(%)	38.29	13.30	.04	.02	.01	.12	.05	.19	16.85	98.01
Vigil										
Mean	-.033	-.117	.356*	-.264*	.257*	-.259*	.162	-.125	-.020	-.097
SD	.360	.339	.331	.305	.311	.332	.303	.277	.266	.333
N	19	19	19	22	23	22	19	19	8	8
p(%)	68.97	14.73	.02	.06	.06	.15	3.12	30.39	83.79	43.32

Mean = Average correlation across subjects. \* = Significantly different from zero correlation with the Dunn-Benferoni criterion for computation of twnty correlated t ratios. SD = Standard deviation. N = Number of subjects. p(%) = Exact probability evaluated by zero- $\mu$  t test and expressed in percent. Baseline = Data collected from Bio 8 to Bio 18 (0800 to 0400 or 1 to 21 hours awake). Vigil = Data collected from Bio 20 to Bio 30 (0800 to 0400, corresponding to 25 to 45 hours awake).

**ACTIVITY SCHEDULE FOR FRAGMENTED SLEEP STUDY  
PHASE 1**

	Sunday	Baseline Monday	Baseline Tuesday	Vigil Wednesday	Fragmented Sleep Thursday	Recovery Friday
0000						
0030						
0100						
0130						
0200				Bio-17	Bio-28	
0230				Chore 8	Chore 9	
0300				Bio-18	Bio-30	
0330						
0400						
0430						
0500						
0530						
0600				Bio-19	Bio-31	
0630						
0700				Chore 6	Chore 10	
0730						
0800			Bio-8	Bio-20	Bio-32	Bio-41
0830		Breakfast	Breakfast	Breakfast	Breakfast	Breakfast
0900		Orientation	Watch 2a	Watch 4a	Watch 6a	Watch 8a
0930			Bio-9	Bio-21	Bio-33	Bio-42
1000						
1030						
1100					Bio-34	
1130		Bio-1	Bio-10	Bio-22	Lunch	Bio-43
1200						
1230		Lunch	Lunch	Lunch		Lunch
1300		Aud. Vig. Tng.				
1330						
1400		Bio-2	Bio-11	Bio-23	Bio-35	Bio-44
1430		Chore 1	Chore 3	Chore 7	Chore 11	Chore 13
1500		Bio-3	Bio-12	Bio-24	Bio-36	Bio-45
1530						
1600						
1630		Dinner	Dinner	Dinner	Dinner	
1700						
1730		Bio-4	Bio-13	Bio-25	Bio-37	
1800						
1830		Chore 2	Chore 4	Chore 8	Chore 12	
1900		Bio-5	Bio-14	Bio-26	Bio-38	
1930						
2000		Watch 1a	Watch 3a	Watch 5a	Watch 7a	
2030						
2100						
2130		Bio-6	Bio-15	Bio-27	Bio-39	
2200						
2230						
2300		Bio-7	Bio-16	Bio-28	Bio-40	
2330						

Fig. 1. Experimental protocol used in this study. A period of sleep is shown shaded. For this study, data from data collection sessions of Bio-8 through Bio-18 are used as the baseline, while data from Bio-20 and Bio-30 were used to represent a period of vigil, sleeplessness of 45 hours. FRT=Fitts reciprocal tapping task. MAST=Memory and Search Task. Aud. Vig.=Auditory Vigilance Test. Watch and Chore represent time periods used for extensive task performance.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) (U). A vigil of up to 45 hours does not destroy circadian cycles in physiological and subjective mood scales, but some rhythms in task performances disappeared with vigil. Vigil does, however, alter some basic parameters of circadian rhythmicity. It tends to reduce strength of rhythmicity and most importantly, to create lengthening of 95% confidence arc for acrophase angles, resulting in appreciably larger 95% confidence ellipse. Thus, major altering effects of vigil of two nights seem to be in a greater scatter of		

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acrophase angles among the individual subjects. Such a greater scatter in time of minimum and maximum due to vigil would result in reduction of amplitude when averages across subjects were plotted along time of day, and also in failure to find a significant cosinor for a group, even though individual subjects might have somewhat reduced but still significant rhythmicity. Relationship between oral temperature and subjective mood and pulse seems to continue undisturbed during the vigil, but correlation between oral temperature and task performance did not survive a process of remaining awake. Findings suggest that more attention must be paid to individual susceptibility to vigil in circadian cycle study.

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